HEAVY METAL PROSTHETIC GROUPS AND ENZYME ACTION

WARBURG

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PREFACE

EVER since it has been known that cells respire the chief problem connected with respiration has been to determine which part of the living matter is autoxidizable. If the combustible substances in the cell are not autoxidizable, and if the cell material itself is not, with what then does the molecular oxygen, which is absorbed by the respiring cell, react?

The answer to the problem lies in the autoxidizable ferrous iron complex which is oxidized to ferric iron by molecular oxygen and transformed again to ferrous iron by the reducing action of the cell constituents.

The long road at the end of which this explanation lay started with the experiments of Edmund Davy, who in 1820 discovered the oxidizing action of finely divided platinum—the first model for cell respiration. In 1857 Claude Bernard observed that after cyanide poisoning, venous blood was bright red, and he thus discovered the specific inhibition of cell respiration by cyanide. In 1885 MacMunn using the spectroscope reported in all cells 'from Echinoderms to man throughout the animal kingdom' the presence of histohaematins and he noted their oxidizing actions. In 1896 John Haldane observed by colorimetric analysis of carbonylhaemoglobin that the carbon monoxide appeared to be more firmly bound in winter than in summer and so discovered the photochemical dissociation of carbon monoxide-iron compounds. In 1923 the oxidation of amino-acids adsorbed on blood charcoal was discovered. This was 'iron catalysis on surfaces', catalysis, which like cell respiration, could be non-specifically inhibited by narcotics and specifically by cyanide. In 1926 the discovery was made that carbon monoxide inhibits cell respiration and that light diminishes this action. In 1928 the absorption spectrum of the oxygen transporting enzyme was determined using light of different wavelengths on the carbon monoxide inhibited reaction.

These are the most important stages, the seven pillars on which our knowledge is based. No one who has seen these

experiments will question the existence of oxygen transporting iron—the enzyme which has contributed more than any other to the explanation of life, but which has as yet not been isolated.

In addition to oxygen transporting iron I have also dealt with the oxygen transporting copper complex of the phenol oxidases, the hydrogen producing iron catalyst of the butyric acid bacteria, the heavy metal of yeast zymohexase discovered in 1942, and finally, the heavy metal of the chloroplasts and its function in the photo-reactions discovered in 1944.

Thus it can be recognized that oxygen transporting iron has not only provided the solution to a great biological problem, but is also the key to the understanding of further and no less important aspects of life.

The last chapter does not deal with heavy metals. It is a report on experiments which I finished in the Spring of 1945—the swan song of the Kaiser-Wilhelm-Institut für Zellphysiologie.

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Berlin-Dahlem, December 1946

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CHAPTER I

DAVY'S MODEL

1. Edmund Davy's experiments

In 1820 Edmund Davy† found that alcohol in aqueous solution is oxidized at room temperature to acetic acid by atmospheric oxygen when a small amount of finely divided platinum is added to the solution. This discovery aroused much interest, as the platinum was reacting under conditions corresponding to those of acetic acid fermentation. Since then, finely divided noble metals have been used as models for oxidizing enzymes and much work has been done in investigating the analogy with biological oxidations. The scientific instinct of such investigators as Berzelius,‡ Schönbein,§ and Bredig‖ persuaded them to regard such an analogy as being more than coincidental. Heavy metal catalysis at surfaces, brought about in model experiments by platinum and in biological processes by iron, has finally been the answer to their questions.

2. Wieland's experiments on Davy's model

In 1912 Heinrich Wieland†† began an investigation of Davy's model with a view to explaining the chemical mechanism of the oxidizing action of platinum. He found that the reaction took place in two stages. In the first stage the metal combined with the hydrogen of the substrate. In the second, molecular oxygen oxidized the hydrogen combined with the metal. If we consider, for example, the oxidation of hydroquinone by molecular oxygen and palladium, then according to Wieland the mechanism would be

$$\text{hydroquinone} + \text{Pd} = \text{quinone} + \text{PdH}_2$$
$$\text{PdH}_2 + \text{O}_2 = \text{Pd} + \text{H}_2\text{O}_2.$$

† Edmund Davy, *Phil. Transact. Royal Soc.* London, 1820, Part I, p. 108.
‡ Jakob Berzelius, *Jahresber. über d. Fortschr. d. phys. Wiss.* **15**, 237 (1836).
§ Christian Friedrich Schönbein, *Journ. prakt. Chem.* **89**, 22, 323 (1863); **105**, 198 (1868).
‖ Bredig *et al.*, *Z. f. physikalische Chem.* **31**, 258 (1899); **37**, 1, 448 (1901).
†† Heinrich Wieland, *Chem. Berichte*, **45**, 484 (1912); **46**, 3327 (1913).

The second of these reactions, the union of hydrogen and oxygen, was known to be catalysed by platinum metals.† Wieland believed that the proof of the first reaction taking place lay in the fact that when he shook hydroquinone with palladium in the absence of oxygen he found that quinone was formed, and that the palladium had combined with an equivalent amount of hydrogen.

In the same way, Wieland showed that many other substances such as glucose could be oxidized by palladium in the absence of oxygen. In this particular case, carbon dioxide as well as hydrogen resulted.

If, however, one examines Wieland's results more closely it is apparent that the oxidations in the absence of oxygen do not proceed beyond the decomposition of a small amount of substrate even when a large excess of palladium is present. Thus 1·8 g. of glucose, after being shaken for 10 hours with 16 g. of palladium, gave only 0·058 g. of carbon dioxide.

Wieland overcame this difficulty with the suggestion that the dehydrogenation was reversible, but it is obvious that this explanation cannot be right in the case of glucose, since the formation of carbon dioxide from glucose is an irreversible process.

3. Personal investigations

In 1924 we applied the manometric technique to the Davy model and were able to study quantitatively‡ the questions which, up till then, had only been dealt with qualitatively. First we investigated the hydrogen–oxygen reaction with palladium. We found that the addition of hydrogen cyanide raised the yield of hydrogen peroxide [found to be only a few per cent. by Traube§] to 60 per cent., and we showed that the equation

$$PdH_2 + O_2 = Pd + H_2O_2$$

expressed the true mechanism of the reaction.

On this basis one should also find hydrogen peroxide in the

† Moritz Traube, *Chem. Berichte*, **22**, 1496 (1889) [p. 1509].
‡ Kanicki Tanaka, *Bioch. Z.* **157**, 425 (1925).
§ Moritz Traube, *Chem. Berichte*, **22**, 1496 (1889).

oxidation of alcohol with palladium, and certainly in the presence of hydrogen cyanide. In no case, however, did we find even a trace of hydrogen peroxide when we shook aqueous solutions of alcohol with palladium.

We concluded, therefore, that the Wieland theory of the Davy model could not be right, and that in the Davy experiment the oxygen had reacted with the metal, the oxidation of alcohol being brought about by palladium oxide. It appears to us that this view is not incompatible with the assumption that it is molecular hydrogen on the palladium which reacts with molecular oxygen since the oxidation of molecular hydrogen and of hydrogen bound chemically to carbon cannot be comparable reactions.

In replying, Wieland† did not accept this point of view. He left unanswered the question as to why hydrogen peroxide is found in the oxidation of molecular hydrogen on palladium and not in the oxidation of alcohol under similar conditions.

4. Experiments of Gillespie and Liu

In 1931, nineteen years after their publication, the Wieland results were re-examined.

Gillespie and Liu‡ reasoned thus. In a saturated solution of hydroquinone, the hydrogen pressure amounts to 10^{-24} atmospheres. On the other hand, palladium forms only a loose combination with hydrogen. Therefore, on thermodynamical grounds metallic palladium cannot dehydrogenate hydroquinone. The quinone formation observed by Wieland must, therefore, have a different explanation.

Wieland prepared his palladium by reduction of the chloride in alkaline solution with formic acid, so that there was a danger of the precipitated palladium containing palladium hydroxide as impurity. In order to avoid this impurity Gillespie and Liu prepared their palladium by another method, namely, by heating palladousammine in a current of hydrogen. Whilst Wieland's palladium immediately formed quinone from hydroquinone in

† Heinrich Wieland and F. G. Fischer, *Chem. Berichte*, **59**, 1180 (1926).
‡ L. J. Gillespie and T. H. Liu, *J. Amer. Chem. Soc.* **53**, 3969 (1931).

the absence of oxygen, no quinone was observed with Gillespie's palladium until oxygen was available. Thus it was shown that Wieland's palladium was not free from oxygen, and that his so-called oxygen-free oxidation was brought about by palladium oxide. This naturally explained the low yields in Wieland's experiments described above.

But there remained unexplained why Wieland found in his oxygen-free oxidations both products of the dehydrogenation, not only the dehydrogenated substrate, but also the hydrogen which had been removed. This question, which was not considered by Gillespie and Liu, is apparently to be answered as follows:

Wieland previously treated with hydrogen the palladium which he used for his dehydrogenation experiments in order to be sure that it contained no oxygen. The hydrogen content of aliquots of the palladium preparation was then determined by heating in a stream of carbon dioxide both before and after the dehydrogenation. The difference gave the hydrogen which had been removed by the dehydrogenation of the substrate.

Let us see how an impurity of palladium oxide would affect the reaction under these conditions. By heating in the carbon dioxide the palladium oxide and the hydrogen would react with one another, and less hydrogen would be found than the preparation originally contained, but after the palladium oxide had been used for a dehydrogenation—for example, the oxidation of hydroquinone to quinone—then on heating in the carbon dioxide less palladium oxide would be available to react with the hydrogen, so that the hydrogen content of the palladium would have apparently increased in the course of the dehydrogenation, and moreover by an amount equivalent to the quantity of substrate which had been dehydrogenated. This follows because the reacting substance was palladium oxide in both the oxidation of the substrate and in the oxidation of the hydrogen.

$$\mathrm{PdO} + \text{hydroquinone} = \mathrm{Pd} + \text{quinone} + \mathrm{H_2O}$$

$$\mathrm{PdO} + \mathrm{H_2} = \mathrm{Pd} + \mathrm{H_2O}.$$

5. Application to biological oxidation processes

The oxygen-free palladium and its oxidizing action were the basis of the well-known 'Theory of Biological Oxidation' which Wieland† advanced in 1912, and which was considered for a decade as offering the solution to the problem of biological oxidation. The oxidation enzymes according to Wieland are not like palladium in being capable of removing hydrogen from the organic substrate, but they activate the hydrogen so that it will react with molecular oxygen. For example, if we let H* represent the activated hydrogen of the substrate, e.g. sugar, amino-acid, or fat, then the general equation for a biological oxidation would be:

$$R\begin{matrix}H^* \\ \diagdown\!\diagup \\ H^*\end{matrix} + O_2 = R + H_2O_2.$$

In 1923 I challenged the theory because in so far as it concerned the reaction mechanism of molecular oxygen it was incompatible with the oxygen transfer brought about by iron.‡ The theory was also incompatible with the oxygen-transporting mechanism associated at a later date with copper and with the yellow enzymes, in fact, with all oxygen-transporting enzymes. When an enzyme transports oxygen the molecular oxygen reacts with the enzyme and not with the substrate. The theory of biological oxidation has proved to be just as erroneous as the conclusions drawn from the palladium experiments which gave rise to it.

Wieland§ has not recanted either in respect of the palladium experiments or of his theory of biological oxidation. He has, however, more and more emphasized that it was immaterial what the oxygen and what the substrate reacted with. All that mattered was that the oxidation of the substrate took place, not by the uptake of oxygen, but by the loss of hydrogen. And so the theory, the object of which had been to define the reaction mechanism of the molecular oxygen, became pointless.

† H. Wieland, *Chem. Berichte*, **45**, 484 (1912); **46**, 3327 (1913); *Ergebnisse der Physiologie*, **20**, 477 (1922); *Liebigs Annalen*, **445**, 181 (1925).

‡ O. Warburg, *Bioch. Z.* **142**, 518 (1923).

§ A. Bertho, *Ergebnisse der Enzymforschung*, **2**, 204 (1933).

Oxidation by dehydrogenation was no theory, but a generally recognized fact which no one would dispute. As far back as 1862 Adolf Strecker† had observed that alloxan and amino-acids react together when in aqueous solution at room temperature. 'Indem das Alloxan durch Aufnahme von Wasserstoff in Alloxanthin übergeht, erfolgt die Oxydation des Alanins und des Leucins zu Aldehyde, Kohlensäure und Ammoniak.' In 1911 Wilhelm Traube‡ had shown that quinone can be used as the dehydrogenator instead of alloxan. And from a biological point of view even more important was the discovery in 1885 by Paul Ehrlich§ that methylene blue in the living cell is converted by the uptake of hydrogen into leuco-methylene blue, acting therefore as an oxidizing agent by removing hydrogen.

If the Wieland theory had been based on nothing more than these results, science would have escaped the ten-year-long polemic over oxygen transporting iron. Oxygen transporting iron and oxidation by dehydrogenation are not incompatible. On the contrary, 'oxidation by dehydrogenation' can almost be regarded as a consequence of oxygen transporting iron, for when molecular oxygen has oxidized the ferro iron to the ferri state it has played its part in respiration. All further oxidations must proceed anaerobically and these oxidations can only be brought about through loss of electrons or loss of hydrogen.

† Adolph Strecker, *Liebigs Annalen*, **123**, 363 (1862).
‡ Wilhelm Traube, *Chemische Berichte*, **44**, 3145 (1911).
§ Paul Ehrlich, *Das Sauerstoffbedürfnis des Organismus*. Berlin, 1885.

CHAPTER II

ACTION OF NARCOTICS ON CHEMICAL PROCESSES IN THE CELL

1. Researches of Claude Bernard

In his lectures on biological processes Claude Bernard† described how fermentation by living yeast cells is inhibited by chloroform and restored on washing out the chloroform. He also observed the same phenomena with oxygen evolution in irradiated living plants. Claude Bernard thereby discovered that narcotics reversibly inhibit chemical processes in the cell.

Claude Bernard also believed that, in contrast to fermentation and carbon dioxide assimilation, oxygen respiration was not inhibited by narcotics. This was an error caused by faulty technique.

2. Limiting concentrations of narcotics

E. Overton‡ determined for a large number of narcotics the concentrations which were just enough to inhibit the movement of tadpoles swimming in water. Overton called these concentrations, which were present in partition equilibrium with the cells, the limiting narcotic concentrations and found, for example, the following values:

	Limiting concentration of narcotic mole/litre
Methyl alcohol	0·57
Ethyl alcohol	0·29
Propyl alcohol	0·11
Butyl alcohol	0·038
Amyl alcohol	0·023
Methylurethane	0·27
Ethylurethane	0·041
Phenylurethane	0·0006
Acetone	0·26
Methylpropylketone	0·019
Methylphenylketone	0·0009

† Claude Bernard, *Leçons sur les phénomènes de la vie, communs aux animaux et aux végétaux*, Paris, 1885 (pp. 276–9).

‡ E. Overton, *Studien über die Narkose*, Jena, 1901.